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Selection of Targets and Assays for High Throughput Screening (HTS)

Co-chairs:

Kate Johnston, Ph.D.

Tim Zacharewski, Ph.D.

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Critical Constituents and Targets

What are the critical cellular constituents or pathways that are likely to be linked to an *in vivo* toxic response?

Guiding principals:

- **Initial holistic approach:**
  - **Apical function > pathway > isolated target**
  - **Function (phenotypic) > cellular pathway > target**
- **Integrate transcript profiling to guide selection of relevant pathways & targets**
- **Multi-dose, multi-time point assays required**
- **Whenever possible, test metabolites & explore cell backgrounds with metabolizing capabilities e.g., MCL5**

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Critical Constituents and Targets

Guiding Principals

- **Select most physiologically-relevant model:**
  - **Organisms, (worms and fish): feasible for HTS but relevance to mammalian systems is problematic**
  - **Tissues: not feasible for HTS**
  - **Co-cultures: intriguing, EXPLORE**
  - **Primary cultures: technically challenging, but preferred; use both rodent and human when possible, possibly stem or progenitor cells**
  - **Cell lines: non-transformed, screen both human and rodent**

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Critical Constituents and Targets

All including:

- **- Apoptosis**
- **- Proliferation (PCNA, Ki67, centriole number)**
- **- Oxidative stress**
- **- Mitochondrial activity**
- **- Receptor interactions (nuclear and membrane)**
- **- Metabolism**
- **- Signal transduction**
- **- DNA damage**
- **- Cytoskeletal/differentiation**
- **- Lipid metabolism and transport channels**
- **-Transporters**
- **- Chromatin remodeling**
- **- Necrosis/membrane integrity**
- **- Cytokines**
- **- Cell-cell communication**
- **- Adhesion**

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Endpoints for Carcinogenesis

Which pathways are the most important to study to understand the pathways leading to cancer?

- **Function first: phenotypic assays for cellular proliferation, death and environmental response**
- **Critical pathways for these functions:**
  - **Apoptosis**
  - **Proliferation, cell-cycle control**
  - **DNA damage and repair**
  - **Chromatin remodeling**
  - **Signal transduction modulation**
    - **SAPKs, MAPKs, Inflammatory response**

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Endpoints for Carcinogenesis

Apoptosis

- Models
  - Rodent: Rat1, NIH 3T3, primary hepatocytes
  - Human: HepG2, TK6 (p53 WT& its $p53^-$ pair), MCL5
  - +/- p53 pair
- Measure both pro-apoptotic & inhibitory activities
- Assays:
  - Early & late markers of apoptosis
  - Multi-dose & multi time points

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Endpoints for Carcinogenesis

Apoptosis assays:

- Cytochrome C release
- AIF release
- annexinV binding
- Caspase activity: 3,7,8,9
- Mitochondrial membrane potential
- Nuclear fragmentation

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Appropriate Endpoints for Carcinogenesis

Proliferation, Cell-cycle control

- **Models**
  - Rodent: Primary hepatocytes, MEFs,
  - Human: HepG2, human foreskin fibroblasts, MCL5
- **Assays**
  - Cell number: cell count, nuclei count
  - DNA content: Hoechst intensity, thymidine or uridine incorporation
  - Cell cycle markers: histone phosphorylation, DNA content (2n, 4n), microtubule stability

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Endpoints for Carcinogenesis

DNA Damage

- Models
  - Rodent: L5178Y, CHO
  - Human: HepG2, TK6
- Assays
  - H2AX phosphorylation
  - P53 activation: phosphorylation, translocation
  - Nuclear morphology
  - Micronucleus
  - DNA fragmentation COMET

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Endpoints for Carcinogenesis

Chromatin remodeling

- **Models**
  - Rodent
  - Human
- **Assays**
  - De-repression of CMV-driven GFP expression
  - Excision repair enzyme activity

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Endpoints for Carcinogenesis

Signal Transduction Pathway Modulation

- **Models**
  - Rodent CHO
  - Human HepG2, U2OS
- **Assays**
  - Kinase activity SAPKs, MAPKs : phosphorylation (Abs - imaging, cell ELISAs)
  - Transcription factor activity (reporters), phosphorylation (Abs - imaging, cell ELISAs), translocation and stability (Abs or chimeras - imaging)

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Toxicology - Wish List

- DNA repair assays
- HTS-compatible lipid metabolism assays
- Differentiation methods for stem cells and a mechanism to identify a population
- Fixable probes for mitochondrial membrane potential, oxygen tension, lipid content
- Assays of more than 48 hrs duration
- HTS-compatible hERG assay (ion channel blocking)

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Toxicology - Wish List, continued

Novel assays to measure Toxicologically-significant endpoints in HTS

- Nuclear Receptors
- G-protein Coupled Receptors
- Toll Rc modulation
- TRKs (growth factor rc)
- Cell backgrounds with enhanced metabolizing capability
- Notch signaling assays
- Card-carrying toxicologists responding to the RFA

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Assay Development

- Assay Development for High Throughput Molecular Screening (R03/R21)
  Reissue for FY2006 of RFA-RM-05-011
- Mark Scheideler, Program Director for Molecular Libraries Initiative
  - Mark Scheideler
    301 496-1779
- Opportunity to submit mechanism-focused Toxicology assays to MLSCN

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Assays in the Queue

- Caspase 3/7, 8 & 9 activation
- pGP
- Cell viability
- Heat Shock Protein 90
- GPCRs: Formyl peptide Rc, 5HT1E
- NfκB pathway
- TNF alpha signaling
- Channels: GIRK, voltage gated K^+
- Htt protein aggregation and degradation